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Efficacy of diatomaceous earth and methoprene, alone and in combination, against *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) in rough rice☆

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Ab ac

Combination treatments of diatomaceous earth (DE) (Protect-It®) and the insect growth regulator (IGR) methoprene (Diacon® II) were evaluated against *Rhyzopertha dominica* (F.), the lesser grain borer, on stored rough rice. Application rates of DE and methoprene ranged from 0 to 500 ppm and 0 to 1 ppm, respectively, in 25 treatment combinations. Tests were conducted by exposing 20 adults for 2 weeks at 32 °C and 75% relative humidity on single varieties of long-, short-, and medium-grain rough rice, removing adults, and holding the rice for 8 weeks at the same conditions to collect F_1 progeny. In the absence of methoprene, mortality of exposed adults increased as the concentration of DE increased, but even at the highest rate of 500 ppm, mortality was only 57.5 ± 12% and 58.8 ± 9.7% in long and medium-grain rice, respectively, and 26.3 ± 4.7% in short-grain rice. Mortality of *R. dominica* exposed on short-grain rice was lower than mortality on long-and medium-grain rice at several combinations with 375 and 500 ppm DE. There was also an unexpected increase in adult mortality with the addition of methoprene so that at 1 ppm methoprene and 500 ppm DE, mortality in long-, medium-, and short-grain rice was 77.5 ± 9.0%, 77.5 ± 10.0%, and 58.5 ± 3.0%, respectively. In the absence of methoprene, progeny produced on long- and short-grain rice ranged from 48.0 ± 21.2 to 87.2 ± 9.0, compared to 16.5 ± 5.5 to 33.5 ± 8.6 progeny produced on medium-grain rice. With the inclusion of methoprene there were few progeny produced in any of the treatment combinations, and the overall average was 0.6 ± 0.3. Similarly, with no methoprene the range of insect-damaged kernels (IDK) was 5.2 ± 2.7 to 12.2 ± 3.1%, but with methoprene the overall average was reduced to 1.8 ± 0.2%. While control of *R. dominica* was somewhat limited with DE, the differences among rice varieties seems to indicate that the specific type and possibly variety of rough rice may influence mortality and reproduction of *R. dominica* exposed to DE. With methoprene, progeny production was greatly suppressed regardless of DE concentration, but combining DE with methoprene would give some measure of adult control.

Keywords: Protectant; IGR; Diatomaceous earth (DE); Insects; Control

1. I d c

In 2004, the United States of America (USA) was the third largest exporter of rice, after Thailand and Vietnam (USDA, 2005). In the USA, rice is primarily produced in Arkansas, California, Louisiana, Texas, Mississippi, and Missouri (Moldenhauer et al., 2004). Although rice is not the predominant crop in these states, it still constitutes a significant part of the total agricultural production and storage system. *Rhyzopertha dominica* (F.), the lesser grain borer, is a serious pest of all stored grains, including rough rice. *Rhyzopertha dominica* is a very destructive pest on stored rough rice, and can cause weight loss through feeding damage and potential loss in milling yield (Cogburn, 1977, 1985; Howell and Cogburn, 2004).

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Diatomaceous earth (DE) is an inert dust that has been extensively tested for control of stored-grain insect pests (Subramanyam and Roesli, 2000). The efficacy of commercial formulations has been documented for a number of insect species on various stored grains, but efficacy often varies with the DE formulation (Subramanyam and Roesli, 2000), and among different commodities treated with a specific formulation (Athanassiou et al., 2003, 2004). Therefore, results on DE efficacy obtained on a particular commodity may not be transferable to other commodities (Athanassiou and Kavallieratos, 2005; Kavallieratos et al., 2005).

Methoprene is an insect growth regulator (IGR) labeled in the USA for direct application to stored grains at 1, 2.5, and 5 ppm. This insecticide is a juvenile hormone analog that interferes with the growth and development of immature insects (Oberlander and Silhacek, 2000). It is effective as a grain protectant against externally feeding stored-grain insects, and also will control *R. dominica* (Arthur, 2004b). *Rhyzopertha dominica* lays an egg outside the kernel and, when the egg hatches, the first instar larva bores into the kernel where it completes development to the adult stage. Once inside the kernel, the developing larva is protected from exposure to contact insecticides. However, contact insecticides can be effective against *R. dominica* because the neonate will be exposed before it enters the grain kernel, and this exposure may be sufficient to prevent development to the adult stage.

Previous tests (Arthur, 2004b) indicated that combination treatments of DE + methoprene effectively eliminated progeny production of *R. dominica* on stored wheat. The objectives of this test were to determine efficacy of DE and methoprene, alone or in combination, against *R. dominica* exposed on three different types of rice, represented by single varieties.

2. Ma e a a d e d

Rice varieties: The rice varieties chosen for the test were Cocodrie, a long-grain variety released in 1998; S-102, a short-grain rice released in 2000; and M-205, a medium-grain rice released in 2004 (Moldenhauer et al., 2004). The classification of rice is usually based on the length-to-width ratio of the kernels; long-grain rice has a ratio of 3.4 or more to 1, medium-grain rice is 2.3 to 3.4 to 1, and short grain is 2.2 or less to 1 (USDA, 1994). Samples of Cocodrie were obtained from the University of Arkansas, (Fayetteville, AR, USA); samples of M-205 and S-102 were obtained from Lundberg Family Farms (Richvale, CA, USA). All samples were from the 2004 crop. Upon receipt in Manhattan, KS, moisture content (m.c.) of the rice samples was determined with a Dickey-John® moisture meter GAC 2000 (Dickey-John Corporation, Auburn, IL, USA), and then adjusted to 13.5–14% m.c., by adding water to the rice samples to increase the m.c. to the desired level.

Grain treatment: The experiment was replicated five times at 2-week intervals. For each replicate, five individual lots of 200 g were weighed out from each rice variety. Individual solutions of 0 (untreated control, water only), 0.25, 0.5, 0.75, and 1.0 mg methoprene/kg (ppm) of grain were prepared in distilled water using a 33.6% active ingredient [AI] commercial formulation (Diacon® II). Each of the individual lots of rice was sprayed using a Badger 100 artists' airbrush (Franklin Park, IL, USA) to mist 0.4 mL of solution from a particular concentration directly onto the rice, a volume rate proportional to the label rate for field application. For each variety and concentration, the treated kernels were put in 0.95-L glass jars and hand-tumbled for 30 s to ensure uniform treatment of kernels with methoprene. After the methoprene was applied, the 200 g of rice was divided into five portions of 40 g each. Each portion of 40 g of rough rice was treated again with Protect-It® 90% DE at rates of 0, 125, 250, 375, and 500 ppm, by placing the 40 g of rice with the DE inside a 0.475-L glass jar, and hand-tumbling for about 1 min. This particular DE was selected based on data obtained in a previous study in which combination treatments of methoprene and Protect-It® were applied to wheat (Arthur, 2004b) for control of *R. dominica*, and a study in which Protect-It® was applied without methoprene for control of the same insect species on rough rice (Chanbang et al., 2007). Also, the amount of 40 g of rice was selected to ensure accuracy of mixing the DE with such a small quantity of rice. Each of the 40 g portions was then divided into two 20 g lots, one of which was placed in a 29-mL vial, and the other was discarded because it was not needed for the test. This resulted in 25 treatment combinations for each rice type.

Twenty, 1- to 2-week-old, unsexed adults of *R. dominica*, obtained from colonies maintained on Cocodrie variety rice in constant darkness inside an incubator set at 27 °C, 60% relative humidity (r.h.), were placed inside the vial for each treatment combination. Colonies were maintained on Cocodrie variety because this particular variety appeared to be very susceptible to *R. dominica*, and therefore suitable for mass-rearing in colonies to provide sufficient insects for testing. All vials were placed in plastic humidity boxes containing a saturated sodium chloride (NaCl) solution to maintain r.h. at 75%, which is equivalent to about 14.0–14.5% grain m.c. (Greenspan, 1977). The experimental procedures used in the present tests to obtain constant humidity levels are described by Arthur (2000, 2002). Three boxes were used, with one box of vials for each rice variety. After the vials were placed in the boxes, each box was placed in a temperature incubator at 32 °C. This temperature was optimum for progeny production of *R. dominica* on wheat (Arthur, 2004b; Vardeman et al., 2006), so 32 °C was selected for this test as well. Temperature and humidity inside the test boxes were monitored with HOBO® data recorders (Onset Computer, Bourne, MA, USA).

After 2 weeks of exposure, *R. dominica* adults were sieved from vials in each treatment combination to assess mortality. Adults were discarded, but rice, dust from feeding damage, and insect frass were returned to the vials, which were placed back in the humidity boxes and into the incubator. After 8 weeks, adult progeny of *R. dominica* in each vial were counted. Insect damage was assessed by sampling 100 kernels for the presence of adult emergence holes (termed an insect-damaged kernel, or IDK).

Data were analyzed by the mixed models procedure (PROC MIXED) of the statistical analysis system (SAS Institute, 2001), with mortality after the 2-week exposures, number of F_1 progeny, and number of IDK as the response variables and replicate as the random effect. Percentage mortality was transformed to angular values (Zar, 1984); number of progeny and number of IDK per hundred kernels were transformed to their square roots to normalize variances. The experimental design was a split–split plot, with rice type as the main plot, methoprene concentration as a sub-plot, and DE concentration as a sub–sub plot. Treatment means were separated at $P<0.05$ using lsmeans (SAS Institute, 2001). Lack-of-fit tests (Draper and Smith, 1981) were conducted using Table curve 2D software (SPSS, Chicago, IL, USA) to determine maximum R^2 of any model which could be fitted to the data set, the R^2 of the selected model, and the R^2 of the selected model as a percentage of the maximum R^2. Regression curves were fitted to the mortality and progeny production data. This approach provides a means of accurately fitting linear and non-linear curves to biological data, and has been used in previous publications concerning the response of insect species to ordered concentrations of insecticides (Arthur, 2000, 2001, 2004b; Chanbang et al., 2007).

Table 1
Equation parameters (mean±SE) for linear and non-linear equations where y = percentage mortality of *R. dominca* after exposure for 2 weeks on long-, medium-, and short-grain rice treated with 0, 0.25, 0.50, 0.75, and 1.0 ppm methoprene (M) combined with 0, 125, 250, 375, 500 ppm of Protect-It® diatomaceous earth (DE) (25 combinations, with concentration of DE as the x term in the equations), on long-, medium-, and short-grain rice (equations plotted in Fig. 1)

ppm M	Rice grain	a	b	Max R^2	R^2	(% Max. R^2)
0	Long[a]	—	0.11	0.70	0.72	97.2
	Medium[a]	—	0.12	0.68	0.70	97.1
	Short[a]	—	0.05	0.70	0.72	97.2
0.25	Long[b]	21.2	0.00022	0.72	0.73	98.7
	Medium[b]	16.2	0.00025	0.72	0.73	98.7
	Short[b]	12.5	0.00011	0.47	0.50	94.0
0.50	Long[b]	22.5	0.00023	0.72	0.77	97.3
	Medium[c]	11.0	0.14	0.74	0.75	98.7
	Short[c]	15.2	0.05	0.70	0.72	97.2
0.75	Long[c]	20.2	0.12	0.77	0.80	96.2
	Medium[c]	18.0	0.11	0.49	0.55	89.1
	Short[c]	11.5	0.06	0.29	0.30	96.7
1.0	Long[c]	13.5	0.12	0.70	0.74	94.6
	Medium[c]	21.5	0.11	0.44	0.48	91.7
	Short[c]	9.5	0.09	0.68	0.69	98.6

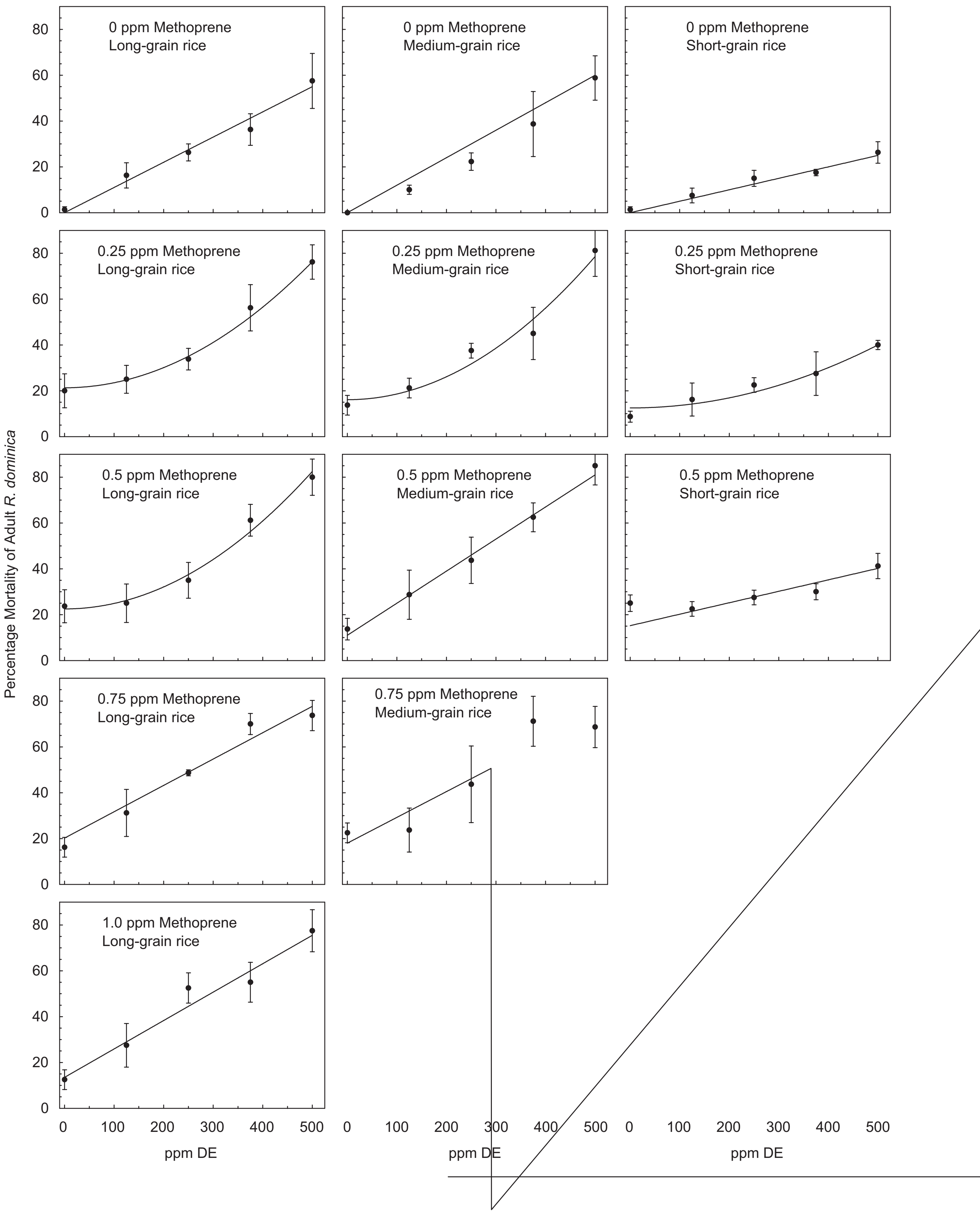
Also shown are the possible maximum R^2 (Max R^2) for any equation fitted to the data, R^2 values of the equations, and R^2 of each equation as a % of the maximum (% Max. R^2).
[a]Linear equations of the form $y = bx$, regression through the origin.
[b]Non-linear equations of the form $y = a+bx^2$.
[c]Linear equations of the form $y = a+bx$, regression through the origin.

3. Re

Mortality of adult *R. dominica* after the 2-week exposure differed among rice type ($F = 16.7$, df = 2, 6; $P<0.01$), methoprene concentration ($F = 18.7$, df = 4, 36; $P<0.01$), and DE concentration ($F = 122.0$, df = 4, 36; $P<0.01$). The only interaction that was significant ($P<0.05$) was between rice type and DE treatment, all others $P>0.05$. Data for mortality with increasing concentration for each rice type and methoprene-DE combination were described by linear and non-linear regression, and regression lines fitted the data for the respective equations (Fig. 1(a–o), Table 1).

With no methoprene, adult mortality at the highest rate of 500 ppm DE was 57.5±12% and 58.8±9.7% in long- and medium-grain rice (Fig. 1a and b), respectively, and 26.3±4.7% in short-grain rice (Fig. 1c). Mortality of *R. dominica* exposed on short-grain rice was lower than mortality on long- and medium-grain rice at the combinations of 0 and 1 ppm methoprene and 500 ppm DE (Fig. 1c and f), and 0.5 and 0.75 ppm methoprene and 375 and 500 ppm DE (Fig. 1i and l). There was a slight increase in adult mortality with the addition of methoprene (Fig. 1d–o), but, even at the combination of 1 ppm methoprene and 500 ppm DE, mortality in long-, medium-, and short-grain rice was only 77.5±9.0%, 77.5±10.0%, and 58.5±3.0%, respectively (Fig. 1m–o).

The number of progeny produced by *R. dominica* after exposure for 2 weeks on the rice differed with rice type ($F = 16.0$, df = 2, 6; $P<0.01$) and methoprene concentration ($F = 445.5$, df = 4, 36; $P<0.01$) but not DE concentration ($F = 2.0$, df = 4, 248; $P = 0.09$). The interaction between rice type and methoprene was also significant ($P<0.01$), but no other interactions were significant. However, the significant differences occurred largely because of the lack of progeny in any of the treatments containing methoprene, regardless of the concentration of DE. With no methoprene, there were fewer progeny in the medium-grain rice than in either long- or short-grain rice at four of the five DE concentrations (Fig. 2). With the elimination of the 0-ppm methoprene treatments and data for DE combined, there was a significant difference with respect to methoprene concentration ($F = 5.5$, df = 3, 19; $P<0.01$) but not rice type ($F = 3.6$, df = 2, 6; $P<0.09$). However, there was no ordered pattern with respect to decreasing survival with increasing concentration of methoprene (non-significant regressions, $P>0.05$), and any differences were assumed to be random. Therefore, all data for progeny in treatments with methoprene were combined, and the average number was 0.6±0.3.



0 ppm Methoprene
Long-grain rice
0 ppm Methoprene
Medium-grain rice
0 ppm Methoprene
Short-grain rice
0.25 ppm Methoprene
Long-grain rice
0.25 ppm Methoprene
Medium-grain rice
0.25 ppm Methoprene
Short-grain rice
0.5 ppm Methoprene
Long-grain rice
0.5 ppm Methoprene
Medium-grain rice
0.5 ppm Methoprene
Short-grain rice
0.75 ppm Methoprene
Long-grain rice
0.75 ppm Methoprene
Medium-grain rice
1.0 ppm Methoprene
Long-grain rice
Percentage Mortality of Adult R. dominica
0
20
40
60
80
0
100
200
300
400
500
ppm DE
ppm DE
ppm DE

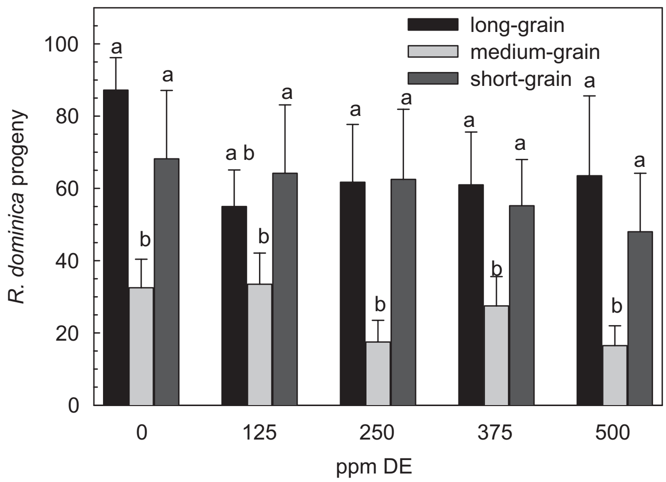


Fig. 2. Number of progeny produced (mean$\pm$SE) by 20 parental *R. dominica* exposed for 2 weeks on three rice types (long-, medium-, and short-grain) treated with 0, 125, 375, and 500 ppm diatomaceous earth (DE) and without methoprene (0 ppm). Rice was held for 8 weeks at 32 °C and 75% relative humidity after parental adults were removed. Means denoted with different lower-case letters are significantly different at $P<0.05$.

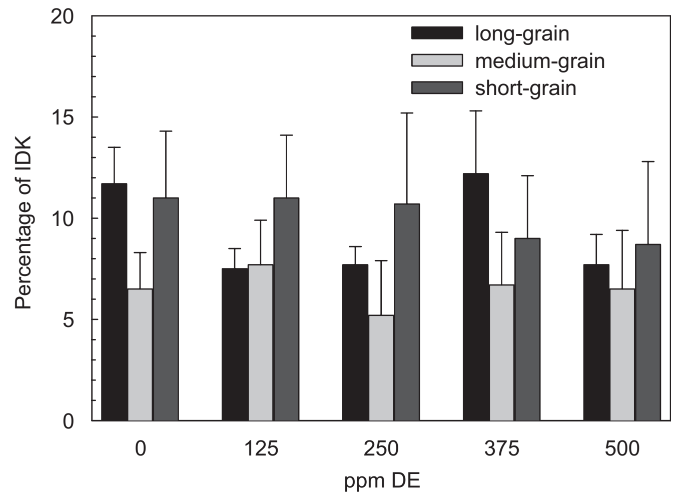


Fig. 3. Percentage of insect-damaged kernels (IDK) (mean$\pm$SE) from exposing 20 parental *R. dominica* for 2 weeks on three rice types (long-, medium-, and short-grain) treated with 0, 125, 375, and 500 ppm diatomaceous earth (DE) and without methoprene (0 ppm). Rice was held for 8 weeks at 32°C and 75% relative humidity after parental adults were removed. There were no significant differences ($P\geqslant0.05$) among means for rice type.

The percentage of IDK was not significantly different among rice type ($F=2.3$, df $=2$, 6, $P=0.18$) or with DE concentration ($F=0.5$, df $=4$, 36, $P=0.70$), but was significant for methoprene concentration ($F=462.3$, df $=4$, 248, $P<0.01$). However, this significant difference for methoprene was because of the inclusion of the 0-ppm untreated control in the analysis (Fig. 3). With no methoprene, the percentage of IDK ranged from $5.2\pm2.7\%$ to $12.2\pm3.1\%$. When the 0-ppm methoprene treatments were eliminated from the analysis, there were no significant differences ($P>0.05$) among rice types or methoprene concentrations, and the overall average was $1.8\pm0.2\%$.

4. D c

Several recent tests have indicated considerable variation in efficacy of different stored-product beetles, including *R. dominica*, among various grain products treated with commercial formulations of DE (Arthur, 2000; Arthur and Throne, 2003; Subramanyam et al., 1994; Arthur, 2004a, b). Mortality of adult *R. dominica* in this test was lower than in previous tests in which *R. dominica* were exposed to hard red winter wheat treated with similar concentrations of DE (Vardeman et al., 2006). Physical and chemical characteristics of the rice hull may account for some of this differential susceptibility with DE, and movement patterns of *R. dominica* may also be different on rice treated with DE compared to movement on other grain commodities.

The results of this test indicated that mortality of *R. dominica* was lower on short-grain rice than on long- or medium-grain rice. Observations with a simple compound microscope showed that the short-grain rice used in this study (S-102 var.) had trichomes, hair-like structures on the surface of the palea and lemma, whereas medium grain (M-205 var.) and long grain (Cocodrie var.) did not. The presence of these trichomes could have either impeded adherence of the DE to the rice kernel or affected movement of *R. dominica*. In addition, mortality was also lower on the long-grain rice variety used in this test, Cocodrie, compared with that from a previous test with the long-grain variety XL-6 (Chanbang et al., 2007).

Rhyzopertha dominica is considered to be one of the most difficult insects to control with DE, compared to other stored-product insects, primarily because adults are slow-moving, are larger than some of the common external feeders in stored grains, and are not as pubescent as the more susceptible species (Quarles, 1992; Fields and Korunic, 2000; Subramanyam and Roesli, 2000). In other direct application studies with stored grains, exposure intervals of 1 week or less usually produced complete mortality of exposed parental adults of *Oryzaephilus surinamensis* (L.), the sawtoothed grain beetle, *Tribolium castaneum* (Herbst), *Sitophilus oryzae* (L.), the rice weevil, and *Sitophilus zeamais* Motschulsky, the maize weevil, exposed to 300 ppm of DE (Arthur, 2000, 2001), and gave reductions in F_1 progeny compared to untreated controls (Arthur, 2002; Arthur and Throne, 2003). In studies with commercial European formulations of DE applied to different commodities, (Athanassiou et al., 2003, 2004) showed that adult *T. castaneum* are more difficult to kill with DE than *S. oryzae*, and can be comparable to *R. dominica* in tolerance to DE. However, all stages of *T. castaneum* are exposed to DE, and it usually causes less damage to stored grains compared to *R. domini*ca.

The variation and difference in efficacy of DE among different grain commodities has been noted in a series of recent studies involving tests with different DE products on different grains (Subramanyam and Roesli, 2000; Athanassiou et al, 2003, 2004; Vayias and Athanassiou, 2004; Arthur, 2004a; Athanassiou and Kavallieratos, 2005; Kavallieratos et al., 2005). Because of these differences in efficacy, combination treatments of different control strategies with DE, such as insecticides (Subramanyam and Roesli, 2000), fungal pathogens (Lord, 2001), and heat treatments (Dowdy, 1999) have been advocated to increase the efficacy of DE in practical insect pest management. In our test, the addition of methoprene eliminated progeny production of *R. dominica*, and including DE in the treatment gave some measure of adult control but could not be considered effective. An alternative approach may be to combine the IGR methoprene with a contact insecticide, or by assessing different rice varieties for susceptibility to *R. dominica* and combining varietal resistance with DE treatment, to provide more effective control.

Ac ed e

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